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EXPERIMENTAL PROLONGATION  
OF THE LIFE SPAN \*

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**S** EVEN hundred years ago the English scientist, Roger Bacon (1214-1292?), presented a logical guide for research regarding old age. He appreciated that man had a long span of life compared with most of his domestic animals. Since all animal life inevitably ages, man has a unique opportunity to study the phenomenon. Thus far man has made little progress in such research because human beings have chosen to expend their energies in improving the supposed comforts of living and methods of warfare.

At present man is not only making little use of animals for the study of aging, but he is neglecting important opportunities for controlled and non-injurious experiments upon man himself. In our own nation today, we have thousands of people confined in mental hospitals and other thousands serving life terms in prisons. It would be an easy matter to divide these into groups and study the effects of widely varied diets upon the diseases of old age, upon the deterioration of bones and teeth, and upon the failure in old age of hearing and vision. Such studies

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would introduce some interest in living to all segments of populations being studied. However, we are doing nothing because hospital administrators are too harassed with the daily problems of providing decent care without embarking upon time consuming ventures of begging for research funds, writing projects, attempting to guide individualistic researchers and publishing results. Possibly the dentists will be the first to make use of human beings for such research because dental projects are relatively simple to administer.<sup>1</sup>

A few examples of the many problems that could be attacked easily in institutions are the effect of long continued use of sugar upon old age, the level of calcium needed in the diet to maintain bones, the effects of different levels of fat in the diet, the relative merits of vegetarian versus omnivorous diets, the effect of sterols upon cardiovascular diseases, the effects of the long continued ingestion of alcohol, the effects of diets rich in milk, imbalances in nutrition between trace elements, vitamins, essential amino acids and unsaturated fats and the effects of various levels of dietary protein. At one time we were nearly embarked upon such a program when Dr. Fred MacCurdy was commissioner of mental hygiene but with the passing of the commissioner from the New York scene the vision was lost. Long before our time the biochemist, Otto Folin at Harvard, called attention to such neglected opportunities.

Today I am certain we do not know the real reasons for such well known phenomena as the longer life of women in comparison with men or the shorter mean span of life of physicians in comparison with other people. Both of these problems were discussed a century ago. Thus, one preacher asserted that women lived longer because they worried less than men. In the third edition of the text upon nutrition by James H. Bennet in 1882, the final chapter is devoted to the problem of the shorter span of life of physicians. Even at this date he rejected the common thesis that exposure to contagious diseases shortened the life span of physicians.

In considering research upon aging with animals, these two examples illustrate problems that can and cannot be attacked. It is evident that the problem of sex differences is subject to attack because white rats that die in old age at the end of their second year of life have the same sex differences as man. On the other hand it seems rather difficult to design experiments with rats that would give a conclusive answer to

the problem of the premature death of physicians. It is possible, however, to set up experiments with rats subject to stress, excess medication, poor diets, loss of sleep and excess alcohol to which some human beings are subject.

In considering any animal research one needs to keep in mind two or more basic concepts that have been debated regularly at most conferences concerning aging for many years. In the first place no conclusive answer has been given as to the possibility of aging without disease. Metchnikoff believed this was possible and cited examples such as the short life span of certain insects and what he thought was the natural death, like sleep, of a few older people. From our research of more than a quarter of a century we are convinced that when one lengthens the life span of an animal such as a white rat, he does so by retarding the onset of certain chronic terminal diseases.

In the second place, one finds in the literature a substantial number of articles indicating that the life span of an animal such as the white rat has been increased by feeding a high level of some substance such as vitamin A. Such research would seem to present studies of premature death from deficiencies or chronic toxicity of one group of animals in comparison with a well fed normal group. However, this depends upon one's point of view.

Before embarking upon a brief discussion of animal research, it may be worth mentioning two other fields that have been neglected in the study of nutrition. The first is the ability of animals to adapt themselves to diets that seem injurious in brief studies. Thus, the feeding of oxalate to young rats for a few weeks will waste body calcium but when the young rat is about half grown it overcomes this handicap so that the body utilizes calcium nearly as well in the presence of oxalate as it does in its absence. Hence, much of our worries over the eating of oxalate rich foods such as spinach or rhubarb seem unjustified.<sup>2</sup> Another example also concerns calcium assimilation in old age after long periods of consuming either high or low levels of calcium. It seems as if the body can adapt itself to low calcium levels in old age more effectively if the body has been used to diets low in calcium during the first two thirds of life.<sup>3</sup>

In the second place there is a slowly growing literature upon the vast numbers of new chemicals finding their way into foods and the imbalances that result from taking large amounts of a single substance

such as synthetic vitamins, or trace elements or amino acids. The literature in this field is little known because advertising it does not help to sell special chemicals, vitamins, trace elements or amino acids but there is great need for long time studies to determine the effect of imbalances upon health during the latter half of life.

Finally there is a naive lack of awareness on the part of some investigators using old animals to study gerontology, that such old animals are always suffering from several chronic diseases. Not long ago we gave investigators at Harvard two rare, thousand day old male rats for the study of an endocrinology problem. We prayed that they were aware that these rats probably had bronchiectasis, tumors and several other afflictions of old age.

Only a few years ago at a conference in New York City concerning the effect of the age of parents upon the number and quality of progeny, several investigators reported upon their researches using old mice. No one mentioned the chronic diseases of old age and every one seemed to assume that he was dealing with pure senility uncomplicated by special pathology. Even in the well established relation between mongolism in man and the age of the parents one wonders if chronic diseases may not be involved.

After this general introduction a bare outline will be given of some of our past experience that indicates that old age and the diseases that accompany it can be profoundly modified. After this we will conclude by presenting for the first time newer use of the old method of parabiosis for the study of aging in animals.

This whole discussion will begin and end, with a consideration of growth because the growth rate and attainment of maturity seem to have more effect upon the life span and the diseases of old age than any other variables.

For centuries the biological literature has contained occasional references to the virtues of fasting and slow growth in creating a long and healthy life. Fasting has been deeply involved in many religious practices. The virtues of a monotonous and limited diet have long been recognized in the prevention of overeating.

The first clean cut experiments proving that diseases could be modified profoundly were those of Moreschi<sup>4</sup> in Ehrlich's laboratory about fifty years ago. He found transplanted tumors could not grow in a partly fasted mouse.

For more than a quarter of a century we have studied retarded growth in various species of animals including rats, dogs, trout and insects. In all of these studies we have designed the diets to provide surplus amounts of all dietary essentials but never enough calories. Thus, each animal has had enough vitamins, inorganic elements, essential amino acids and fats but never enough calories for growth.<sup>5</sup>

The common white rat which passes through its normal span of life is very suitable for such studies because it is large enough for physiological studies and not very subject to parasitic diseases. Our poorest results have come from studies with dogs because fasted and retarded puppies are killed much more easily by parasitic diseases such as hookworms, than are normal animals. By the use of special sanitary procedures we have now improved our experimental techniques with dogs and hope soon to attempt again a study of retarded growth.

Rats retarded in growth by the above procedures and kept for the whole of life exhibit certain profound changes in their span of life and the diseases of old age. In the first place the span of life is lengthened substantially. Large numbers of rats can be produced that exceed a thousand days of age and some survive to 1400 days, or more than twice the mean span of life. Hence, man has it within his power to predetermine and lengthen the life span of one species of animals. Can this be true for all species?

In a normal group of rats, the females tend to live longer than the males. In retarded animals this is not true. They have equal spans of life. The growth retardation makes the male equal to the female in its long span of life. Possibly a study of this phenomenon will unravel the mystery of the longer survival of the human female.

In the second place our own studies and those of others have confirmed the early observations of Moreschi that the development of tumors is influenced profoundly by the retardation of growth. Normally about 12 per cent of our rats are afflicted with tumors of spontaneous origin at the time of death in old age. These retarded rats that survive much longer and usually die suddenly from lung diseases develop few tumors.

In the third place these retarded rats have certain parts of their bodies that seem to age at the normal rate and other organ systems that age more slowly. The bones seem to age at a normal rate and become very fragile in animals that are retarded and survive to 1400 days. The

eyes seem to fail at about the same rate but the individual variability is so great that it is difficult to measure. In contrast the hair of a retarded rat resembles the fine fur of a young animal. The skin of a retarded animal appears like that of a young one but when one cuts it one finds the tough characteristics of age.

A few problems concerning retarded growth have been given attention in our laboratory for many years but have never yielded conclusive results. A number of attempts have been made to determine if the learning ability or emotional stability of the retarded rat is superior to the normal. Most results have been inconclusive if the testing technique did not involve some reflection of a handicap from a chronic disease. In other words it seems that the brain of a normal old rat retains its functioning ability better than the lungs or reproductive organs.

In a recent unpublished report by a graduate student in psychology, Robert Ader, he concluded: "Although there were no significant differences in the behavior pattern observed, the direction of the differences was consistent, implying greater emotionality of the control animals." He concluded that possibly "the lower level of activity of the more emotional control group was more conducive to the avoidance of errors than was the agitated activity of the retarded group." This seems to say about what we often read in comparisons of the running of machines by seventeen and seventy year old workers, namely that the older worker moves more slowly but achieves the same amount, due to the exactness that comes from experience.

One inherent difficulty in psychological testing of retarded animals is that one cannot use motivation from hunger unless he feeds his retarded animals and permits growth. In this field we would like to test the loss of ability to select essential nutrients in relation to age since we found some years ago that the ability to select such essentials is very marked when rats are young but this ability gradually declines with age.<sup>6</sup> However, we have found no satisfactory method of testing retarded rats that are always hungry.

The problem of loss of reproductive powers in both normal and retarded rats has been studied from time to time. In one test some years ago in which males were fed marginal allowances of vitamin E, it was found that these male rats lost their fertility early in life but that this had no effect upon the total span of life. Retarded male rats seem to be permanently sterilized.

Some of the females, however, can be bred after a substantial period of retardation but none have exceeded the normals. In our rat colony the age record for reproduction by a female rat has been 26 months, while the oldest retarded rat that could produce a litter was 17 months. For some years we have been selecting rats from litters born very late in the life of females to determine if this might lead to a strain of rats that could normally attain a greater life span. No results have come from this study thus far.

Another problem of retarded growth concerns the ability of retarded rats to prevent the development of bone tumors. Some years ago we found that the feeding of 5-6 microcuries of calcium 45 per gram of body weight to young rats for a two week period shortly after weaning led to the production of bone tumors in the adult rats starting at about 200 to 300 days of age. All rats given this level developed bone tumors. Below this level a bone tumor is rare.<sup>7</sup>

One can then ask if the retardation of growth in such rats might prevent the development of tumors of bones as it does in soft tissues or if the retardation of growth might prolong the span of life and hence make the bone tumors appear at a much later age from lower levels of Ca45. This experiment was started in the summer of 1955 but has given no results thus far.

In this latest study both hamsters and rats are being used since we know of no retarded growth nor tumor studies of this type with hamsters. The use of several species seems important from our experiences with dogs.<sup>8</sup> Using the same regime of feeding calcium 45 and the same dosage in relation to body weight, we have produced no bone tumors within the first year of life. The Ca45 merely produced thinning of the shafts of the long bones so that some were fractured spontaneously as shown in x-rays. These healed without splinting and these female beagles seem quite normal today in their second year of life.

Hence, there may be marked species differences in the response to changes initiated by radioactive materials when fed to young rapidly growing animals such as rats, dogs, hamsters and other species. Although the margin is wide between the level of radioactive calcium used as a tracer and the level that produces bone tumors, the species variation may be important especially since some Ca45 is being fed to human beings in some laboratories.

Both the hamster and the dog may deserve more extensive use in

research upon aging since the hamster seems to be subject to forms of arthritis in old age similar to those of man and since the dog seems subject to changes in old age similar to those of man such as alterations in the prostate.<sup>9</sup>

Inasmuch as retarded animals never attain normal adult size, but are always somewhat stunted, one may ask if the smaller animal is destined for a longer span of life. This has interest as a human problem since our culture tends to accelerate the growth of children and produce larger adults. In studies of normal rats that have grown as rapidly as possible we have attempted to determine if there is a relationship between ultimate body size and total length of life. Contrary to what one might believe there is a very positive correlation between size of body attained and length of life. We are not aware of similar studies with human beings but they may exist.

Next in importance to retardation of growth as a means of lengthening the span of life of experimental animals is the technique of keeping animals from becoming fat. The life span of rats is lengthened if they are kept thin either by restriction of food intake or by exercise. Even if the rat is allowed to be overweight until middle life and then obliged to reduce, there is a favorable influence upon length of life.

In studies of aging one is faced with the basic problem of whether it is possible to improve health without any lengthening of the span of life. In other words, can one modify a given organ system without change in that one which terminates life in old age. As a concrete illustration one may ask if one can improve the quality of bones in old age without modifying the bronchiectasis that ultimately destroys the animal.

A number of studies with white rats has indicated that one can feed diets of special composition such as liquid milk with the result that the bones remain denser in old age and the teeth show no decay. However, chronic bronchiectasis and other afflictions of later life proceed at their usual rate. Hence, the rat dies after the common mean span of life but has superior bones and teeth at the time of death.<sup>10</sup>

In concluding this discussion a brief review will be given of our experiences during the past three years in exploratory research to determine if the methods of parabiosis may have some use in fundamental studies of aging. In his review of parabiosis, Finerty<sup>11</sup> states that this joining of two rats by surgical operation was first used in Claude Ber-

nard's laboratory by Paul Bert in the 1860's. Through the common capillary circulation the two rats share many but not all of the constituents of the blood.

Our own interests in parabiosis arose in the course of studies upon the decay of teeth. Kamrin<sup>12</sup> made use of parabiosis by feeding one of each pair of rats a given carbohydrate, to learn more about the food factors responsible for decay. He also gave us our initial lessons in surgery for this study.

The first question to be answered was that of the age that could be attained by a pair of rats parabiosed shortly after weaning. Only two pairs were prepared. The older of the two was killed when 527 days of age because one developed a chronic bladder infection and we felt that evidence was adequate for such survival. Such survival is equivalent to 53 years in the case of man.

Only one study of interest was made upon these old parabiosed rats during the latter half of their lives. While these rats were alive, a bacteriologist from Queen Elizabeth College in London, Dr. Rachel White, was making comparative studies of the microorganisms of old and young rats in our laboratory. She had found the greatest contrast in the number of yeasts in the feces of old and young rats fed similar diets. Several studies were made of these parabiosed rats with time intervals of several weeks between samplings. Every test showed consistent differences in the microflora and this difference was maintained. Thus, in spite of living united from an early age and eating from a common food supply, the distribution of microorganisms in the intestinal tract was characteristic for each individual.

The next studies made during the past summer were designed to help secure answers to the classical problem of whether a young animal has substances circulating in its blood that can rejuvenate the old or vice versa, whether the blood of the old can produce aging in the young.

Nearly a century of research indicates that the basic problem of uniting a young and a middle aged or old animal, would probably be due to failure to effect unions due to skin sensitivity. In most parabiotic research, litter mates are used but much has been done in uniting non-litter mates allowing for about two thirds failures.

In the hope of reducing inherited allergies to a minimum and still have rats of quite diverse ages, a series of litters was produced using the same dam and sire. Since we have not learned to make parabiotic

unions between rats of very different sizes, the older rats were retarded in growth for as much as eight months before the operation was performed. Thus, we had available rats of quite different ages, the same size and from the same parents.

Parabiotic unions were made between such rats in July and they are still in good condition now in October. In such unions it looks as if the most important factor is the anesthesia. The reactions of rats of the same size but different ages to the barbiturates seem quite different. In the next place the temperament of the two animals seems important. If two rats are not adjusted to each other, one will chew the head of the other until it is destroyed.

In some cases one meets the problem of "parabiotic toxicity" which has long been recognized by workers in this field. Under such conditions both rats may eat and drink normally but one seems to be drained of certain essentials. The rat that suffers from this drainage shows ears and tail that are pale and blanched while the other has these organs that are very red. During the past summer it has proved possible to take such rats apart and unite the successful one to a new partner.

By the careful use of sedatives over a long period of time it may be possible ultimately to unite rats of very different sizes and ages but this has not been tried thus far.

Since retarded rats age very rapidly after they attain maturity in comparison with normal rats, it may be possible to learn something about the relation of the internal milieu to aging by this method of study. We may even find it possible to test the hypothesis of J. H. Cohausen in his book of 1744 entitled "*Hermippus redivivus*," in which he is said to have advocated that an old man regains some youth by acquiring a young woman.

#### SUMMARY

Studies covering a period of a quarter of a century indicate that the life span of experimental animals such as the white rat can be increased much by the retardation of growth. Under normal conditions the female outlives the male but when the life span is extended there are no differences. Chronic terminal diseases such as tumors are much restricted by retardation of growth. Under normal conditions animals that attain a larger body size have longer spans of life. Early sterility of either sex does not influence the total span of life. Under normal

conditions animals that grow larger tend to have longer spans of life. By certain feeding regimes such as the use of large amounts of fresh milk, the bones and teeth may be better preserved in old age but the span of life is not lengthened because the terminal disease involves the lungs which are not affected by the feeding regimes.

The old technique of Paul Bert of parabiotic union by which pairs of rats are sutured together so that they have a common capillary circulation may have use in the study of aging. By combining the techniques of retarded growth and the production of successive litters from the same parents, it has proved possible to effect parabiotic unions between rats that are 60 and 300 days old. The two most important factors in such operative procedures seem to be the proper dosage of anesthetics for the rats of different ages and the temperaments of the two animals. Maladjusted animals destroy each other. In case of failure one parabiotic rat can be severed from the union and combined with a new mate.

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